MICROBIAL DECOMPOSITION OF FIR LITTER*

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To elucidate the process of microbial decomposition of fallen leaves, it is essential to make clear the relations between the constituents of the leaves and the activities of the major groups of microorganisms taking part in it.

Alcohol-benzol soluble substances, among the constituents of leaves, are said to retard the decomposition, while there is a conflicting view that this fraction disappears quite rapidly. And a high content of water soluble substances is generally considered to offer a favourable condition for the earlier stages of decomposition. Here, the cold-water soluble fraction is decomposed rapidly, but the hot-water soluble fraction tends to be broken down less readily because of the presence of tannin in it.

This note is concerned with an experimental approach to the decomposition of soluble substances in the needles of a fir, *Abies firma*, by the soil microorganisms of a firwood.

I wish to express my gratitude to Professor T. Jimbô, under whose guidance the study was carried out. Thanks are also due to Dr. T. Saitô for his valuable suggestions.

MATERIAL AND METHODS

The needles of *Abies firma* collected in the spring of 1959 from trees in the Aobayama Botanical Garden of the Tôhoku University in Sendai were treated in different ways as follows, after heated at 60°C. for two days in advance : (1) with a mixture of alcohol and benzol (1 : 2) for ten hours, (2) with hot water for four hours, (3) with alcohol and benzol and then with hot water, and (4) the control. 40 g. of them were placed as a mass 5 cm. in diameter and 5 cm. deep in each of two open bottles, and kept at 26°C., after moistened with tap water and inoculated

* To Prof. Tadao Jimbô I dedicate this note in celebration of his 63rd birthday.

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with soil suspension. The soil suspension was prepared by mixing 100 g. of soil from a firwood in the Botanical Garden with 5 litres of tap water. During the course of experiment the water contents of the leaves in the bottles remained from 64 to 68%.

Determination of the numbers of microbes was carried out by plating out on Waksman's acid glucose-peptone agar for fungi and on sodium caseinate agar* for bacteria and actinomycetes.

RESULTS

The course of decomposition by filamentous fungi is given in Fig. 1 in terms of their plate counts, though they are liable to involve the number of conidia without showing the true amount of vegetative mycelia. The fungal growth in the control

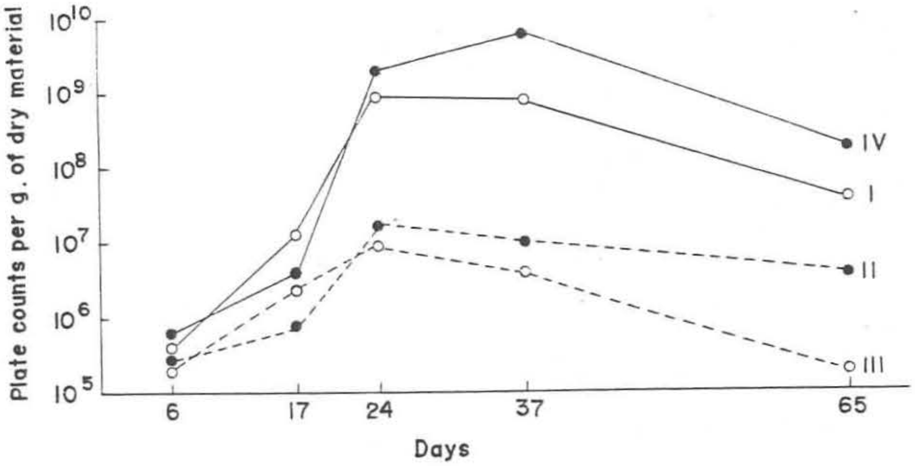

Fig. 1. The number of fungi in fir litter as influenced by deverse treatments : (I) with alcohol-benzol, (II) whith hot water, (III) with both alcohol-benzol and hot water (IV) the control.

(the fourth series) is likely to take a sigmoid curve with a maximum reached 37 days after. In the first series devoid of the alcohol-benzol soluble fraction, the number of fungi increases quite rapidly during the earlier period, though their growth is suppressed to some extent at the very beginning; but the subsequent growth is not so prominent as in the control. In the second and third series both without the water soluble fraction (in the third series the alcohol-benzol soluble fraction being absent in addition), the fungal growth is by far less compared with the above two series. Hence the absence of the water soluble fraction is detrimental for the fungal growth. It is to be noted that a similar relation as between the

* Sodium caseinate 2 g., glucose 1 g., dipotassium phosphate 0.2 g., magnesium sulphate 0.2 g., ferrous sulphate trace, tap water 1000 c.c., agar 12.5 g.

former two exists between the latter two, and this is due obviously to the removal of the alcohol-benzol soluble fraction.

In Fig. 2 are set out the plate counts of bacteria plus actinomycetes.* It will be seen that these two groups of organisms attain the maximum growth later than fungi, and that the second and third series deprived of the water soluble fraction, whether with or without the alcohol-benzol soluble fraction, show, despite their

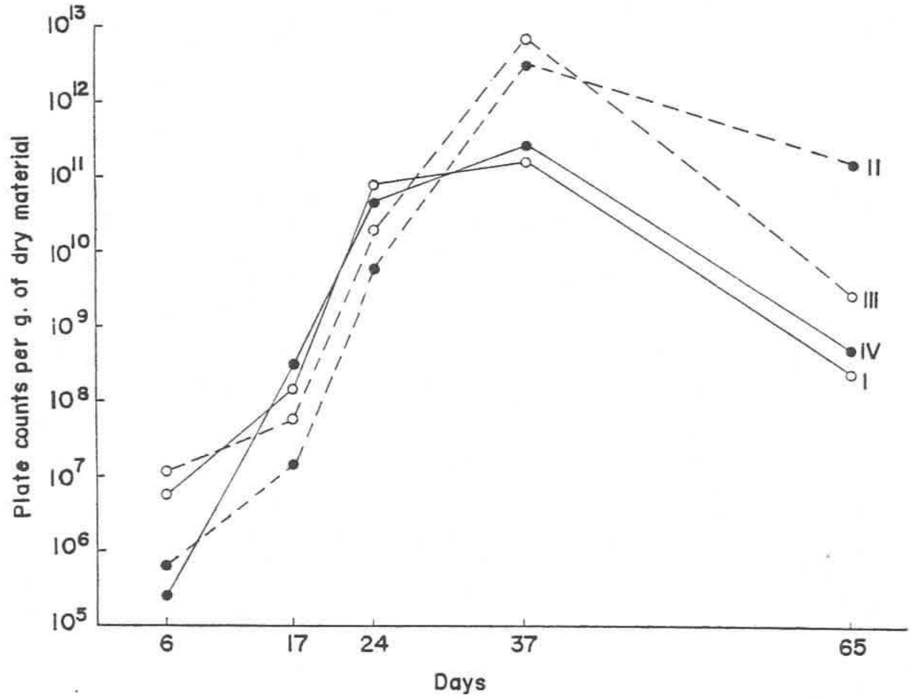

Fig. 2. The number of bacteria plus actinomycetes in fir litter as influenced by diverse treatments: (I) with alcohol-benzol, (II) with hot water, (III) with both alcohol-benzol and hot water, (IV) the control.

rather slow growth at first, a better growth of bacteria and actinomycetes in the long run due possibly to the release from the competing fungi. The removal of the alcohol-benzol soluble fraction is admittedly beneficial to bacteria and actinomycetes at the beginning of decomposition. It is likely, however, that, in all events, a vigorous growth of fungi suppresses the bacterial and actinomycetous growth.

The rate of decomposition in the course of 70 days was estimated in five lots of 100 leaves by the reduction of dry weight on the basis of diversely treated leaves, and it was found to be 29, 25, 20 and 15% in the fourth (the control), first, second

* It should be emphasized that the values shown by dots on curves in both Figs. 1 and 2 are the means of two, virtually very close values obtained in duplicate bottles.

and third series respectively. This order is the same as in the fungal counts (see Fig. 1). On the other hand, the pH value of leaf infusion seems to be indifferent to the decomposition of litter in the scope of the present experiment (pH 5 or so at the beginning and nearly pH 7 at the end).

From the foregoing facts the following conclusion may be drawn :

(1) *Removal of the alcohol-benzol soluble fraction stimulates the growth of fungi at the beginning of decomposition, and this is true also of bacteria and actinomycetes.*

(2) *Removal of the water soluble fraction is detrimental to the growth of fungi in particular.*

(3) *The fungal growth is primarily responsible for the decomposition, and consequently exerts a more or less influence upon the bacterial and actinomycetous growth.*

The fungus flora identified on the plates (based on Gilman 1950 and Thom & Raper 1945, 1949) and the abundance of each species are listed in Table 1. It will be seen that a few species are excluded by washing with hot water, and that penicillia are by far predominant in the variety and number, *Penicillium funiculosum* being ubiquitous and dominating as a rule. *Rhizopus nigricans*, the only phycomycetous species here, flourishes only in the earlier period of decomposition, while *Trichoderma koningi* becomes prominent later on.

Here I would like to refer to the previous findings by other authors relative to what is stated above.

As to the influence of the hydrogen-ion concentration, Melin (1930) revealed that it does not exert any decisive influence upon the rapidity of decomposition of the leaves of a variety of trees.

The water content of 64 to 68%, that the leaves showed in the present experiment, falls within a range of 200 to 300% of dry litter, which, according to Nakayama (1956), is more favourable to fungi than to bacteria in the litter of *Pinus densiflora*, where more water stimulates the bacterial growth.

It is said that a high content of the alcohol-benzol soluble fraction of the needles causes them to be more slowly decomposed than the deciduous leaves ; and Melin (1930), Ohmasa & Mori (1937), Tsutsumi (1956) and others pointed out that needles of various kinds are occasionally almost twice as rich in this fraction as in the deciduous leaves. Nevertheless, Shibamoto & Nakajima (1951) reported that it diminishes with the progress of decomposition of the leaves in the Ao-layer, and Saitô (1957) showed that is disappears rapidly during the earlier period of decomposition of beech litter under the natural conditions. And Melin (1930), Waksman & Tenney (1927), Nakayama (1956) and Tsutsumi (1956) noticed that this fraction is not always so resistant that needles rich in it are more slowly decomposed than the deciduous ones. According to Tsutsumi, the alcohol-benzol soluble fraction in the leaves of *Pinus densiflora* and *Cryptomeria japonica* does

Table 1

Plate counts of fungus species ($\times$ 10^4) per g. of material

	Days	6	17	24	37	65
Series I	*Rhizopus nigricans*	4	530	48000		
	Trichoderma koningi	9				1200
	Aspergillus luchuensis			6000		
	Penicillium frequentans		500			
	P. purpurrescens		510			
	P. spinulosum		82			
	P. aureo-violaceum	6			18000	
	P. charlesii			6000	18000	
	P. nalgiovensis		30	13500		
	P. roqueforti ?				18000	
	P. funiculosum	1	15	24100	18000	2400
	P. purpurogenum	2	24		17000	300
	Other penicillia	1	7			
	Pullularia pullulans	5	15			
Series II	*Rhizopus nigricans*		21	541	18	
	Trichoderma koningi	2	3	120	115	19
	Penicillium frequentans	2	9			
	P. purpurrescens	13	3			
	P. spinulosum		1		150	
	P. aureo-violaceum	6		30		
	P. nalgiovensis		3	150	10	
	P. funiculosum	6	42	810	640	348
	P. purpurogenum		5			
	Other penicillia				9	
	Pullularia pullulans	2				
Series III	*Rhizopus nigricans*		9	500	30	
	Trichoderma koningi		30	25		4
	Aspergillus luchuensis			30		
	Penicillium frequentans	3	110			
	P. purpurrescens		30			
	P. spinulosum		10		60	
	P. aureo-violaceum	7			108	12
	P. nalgiovensis	3				
	P. funiculosum	2	69	680	180	
	P. purpurogenum	1				
	Other penicillia			75		
	Spicaria sp.		30			
	Pullularia pullulans	9				
Series IV	*Rhizopus nigricans*	26	54	105000		
	Trichoderma koningi	2	42			15800
	Aspergillus luchuensis			30000	18000	
	Penicillium frequentans	3	58	1500	120000	
	P. purpurrescens		100			
	P. spinulosum		15		78000	
	P. aureo-violaceum	12			72000	
	P. charlesii			480		
	P. nalgiovensis	1	30			
	P. roqueforti ?				3600	
	P. funiculosum		155	1500	90000	3600
	P. purpurogenum	2	24	25000	36000	1800
	Other penicillia	2	9	28000	39600	1800
	Pullularia pullulans	15	9			

not actually retard the decomposition of the leaves as a whole, but the fraction itself is not decomposed rapidly at first.

As regards the fir litter here in question, the alcohol-benzol soluble fraction present abundantly in the leaves seems not to affect their decomposition remarka-

bly, as has been described above.

It has been mentioned above, on the other hand, that the removal of the water soluble fraction suppresses the fungal growth a great deal, and ultimately permits a pronounced growth of bacteria and actinomycetes.

In this connection Melin (1930) pointed out that variation in the content of water soluble constituents and of lignins may to a certain extent exerts an influence upon the capacity of decomposition within one and the same species, and that the content of water soluble matter affects the rate of decomposition only during the first weeks as a rule.

And, there is also a view that the content of inorganic matter of the litter is relevant to some extent to its decomposition.

SUMMARY

1. The leaves of *Abies firma* killed by heat and washed with alcohol plus benzol, with hot water, or with both alcohol-benzol and hot water were incubated after inoculated with a suspension of soil of firwood. Plate counts were made of fungi and bacteria plus actinomycetes from time to time.

2. Removal of the alcohol-benzol soluble fraction stimulates at the beginning of decomposition the growth of fungi and also of bacteria and actinomycetes.

3. Removal of the water soluble fraction is detrimental to the growth of fungi in particular.

4. The fungal growth is primarily responsible for the decomposition, and as a consequence affects the bacterial and actinomycetous growth.

5. The fungus flora is dominated by a variety of penicillia.

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